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Molecular characterization of Russian wheat aphid consumption by spiders in winter wheat

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Abstract. Accurate characterization of predator-prey linkages in agroecosystems is important prior to the implementation of conservation biological programs. The Russian wheat aphid, *Diuraphis noxia* (Hemiptera: Aphididae), is a significant pest of wheat and barley in the United States. This research utilized molecular gut-content analysis as a minimally disruptive technique to characterize the trophic connectivity between two spider species, *Tetragnatha laboriosa* Hentz 1850 and *Pardosa sternalis* (Thorell 1877), and *D. noxia*. We describe the development of species-specific primers that amplify a 227 bp fragment of *D. noxia* COI mtDNA to identify the frequency of predation under varying aphid densities and developmental stages of winter wheat. We tested the hypotheses that predation rates on *D. noxia* would be highest for both spider species at the greatest aphid infestation level in the aphid-resistant wheat cultivar plots and that densities of *T. laboriosa* would be highest at the highest aphid infestation level in the aphid-resistant cultivars. Despite short detection periods of prey DNA in the laboratory, 32% and 48% of field-collected *T. laboriosa* and *P. sternalis* spiders screened positive for *D. noxia* DNA, respectively. *T. laboriosa* densities were highest at the highest aphid infestation level. Aphid-resistant wheat cultivars did not impact predation rates or densities. Additionally, *P. sternalis* predation on *D. noxia* increased with increasing aphid infestation levels. Given the high predation rates on *D. noxia* and their association with increased aphid densities, both spider species represent important natural enemies within wheat agroecosystems, and further research is required to quantify their impact on aphid populations.

Keywords: *Diuraphis noxia*, biological control, predator-prey interactions, molecular gut-content analysis, generalist predators

Given the premise that natural enemies, with diverse modes of foraging and asynchronous life cycles, act as a whole to limit pest populations as opposed to individual species acting alone (Sunderland et al. 1997), it is essential to understand the behavior of all constituent parts of the community. Importantly, many are often present before pests arrive and thereby impact pests during colonization (Edwards et al. 1979; Chiverton 1987; Landis & van der Werf 1997; Harwood et al. 2004). Given this attribute, characterizing their feeding behavior in agroecosystems forms an important component of developing pest management approaches. However, understanding the trophic interactions between predators and prey can be complex. Observations of predator-prey interactions are often disruptive to the study system and inherently biased in terms of what can be "observed" and the time of sampling. In the last 20 years, molecular techniques alleviated many of these concerns and have contributed to the understanding of trophic relationships in the field (reviewed by Symondson et al. 2002; Sheppard & Harwood 2005; Weber & Lundgren 2009).

The Russian wheat aphid, *Diuraphis noxia* Kurdjumov 1913 (Hemiptera: Aphididae), is a pest of wheat, *Triticum aestivum* L. (Poales: Poaceae), and other small grains in all wheat-growing countries except Australia (Elliott et al. 1998). Aphid-resistant cultivars that prevent the wheat leaf from curling are now widely planted, which will likely expose aphids on the plant surface (Hawley et al. 2003). Thus, external disturbances like wind, rain, and predators (von Berg et al. 2008) could trigger a higher falling rate than susceptible cultivars, thereby increasing contact with epigeal predators. However, the use of resistant cultivars has been compromised by the introduction of Russian wheat aphid biotype 2 (Haley et al. 2004), creating a further need to consider epigeal predators for pest management. Spiders are a major component of this fauna (Sunderland & Greenstone 1999), aggregate to areas of high prey density (Harwood et al. 2001, 2003), and feed on a variety of crop pests, including aphids (e.g., Chiverton 1987; Sunderland et al. 1987; Winder et al. 1994; Harwood et al. 2004, 2005; Oelbermann & Scheu 2009). Additionally, the high falling rates of aphids from wheat plants (Kerzicnik et al. 2010) suggests that if predator densities are sufficiently high, both epigeal and web-building spiders could exert some degree of control. Identifying the foraging behavior of such species is therefore required in order to determine the potential roles of these species for biological control.

Tetragnatha laboriosa Hentz 1850 (Araneae: Tetragnathidae) is a dominant predator within several agroecosystems (Young & Edwards 1990; Nyffeler & Sterling 1994) and can rapidly recolonize following disturbance (Howell & Pienkowski 1971) by means of ballooning throughout their lifetime (Bell et al. 2005). It builds small webs, capturing many aphids (Culin & Yeargan 1982; Nyffeler & Sterling 1994; Jmhasly & Nentwig 1995) and small flies (Provencher & Coderre 1987). Spiders in the genus *Pardosa* (Araneae: Lycosidae) are also commonly found in agroecosystems (Marshall & Rypstra 1999; Samu & Szinetár 2002; Öberg & Ekbom 2006), and *Pardosa sternalis* (Thorell 1877) is particularly common in northern Colorado and, as with most epigeal predators, *Pardosa* are affected by plowing, tillage and mechanical weed control (Thorbek & Bilde 2004). They are active prey hunters, have a broad feeding niche (Bailey & Chada 1968) and impact pest species at a different stratum in the crop.

Since *T. laboriosa* is a known aphid predator and both spider species are dominant in Colorado wheat, it would be

expected that these two spider species feed on *D. noxia* in the field. For this study, the following hypotheses were examined: 1) *T. laboriosa* densities will be highest at the highest aphid infestation level in aphid-resistant wheat cultivar plots and 2) *T. laboriosa* and *P. sternalis* predation on aphids will be highest at the highest aphid infestation level in aphid-resistant wheat cultivar plots. Using PCR with species-specific primers, the goal of this study was to measure the frequency with which *T. laboriosa* and *P. sternalis* prey on *D. noxia* in a winter wheat agroecosystem in order to identify their potential role in biological control.

METHODS

Study Site and Planting Regime.—Research was conducted in winter wheat at the Colorado State University Agricultural, Research, Development and Education Center (ARDEC), Fort Collins, Colorado, USA, (GPS coordinates: 40.65099°N, 104.99671°W; elevation 1534 m). The site was irrigated once prior to planting on 3 September 2007 to ensure uniform plant emergence, and wheat was grown according to standard agronomic practices for the region. The wheat (cultivar STARS 02RWA2414-11/5*CO00554) was planted on 11 September 2007, and sampling occurred during the 2008 growing season. No herbicides were applied during the experiment.

Experimental Design.—This study was a split-plot design with repeated measures. The whole-plot factor replicated eight times was aphid infestation level, and the split-plot factor was the infestation level of aphids in wheat cultivars. Split plots were 3.24 m^2 with six wheat rows, and "Hatcher" wheat was planted as a buffer between and outside of the plots. There were three aphid infestation levels (0×, 1×, and 10×) to examine predation under varying aphid densities. Within each plot, winter wheat plants were infested with greenhouse-reared (L16:D8 cycle, 24 °C, 65% humidity) *D. noxia* biotype 2 using a Davis inoculator (Davis & Oswalt 1979). Four, 1-m rows in the center of the 1× and 10× plots were infested with approximately 246 and 2,460 biotype RWA2 aphids, respectively, on 7 March 2008. No aphids were added to the 0× infestation level plots. Infestation numbers to be applied in the field were estimated by using the Davis inoculator to deliver aphids to 10 Petri dishes. The number of *D. noxia* per inoculator delivery per Petri dish was averaged, providing an estimate of the number of aphids delivered to wheat in the field. Two different wheat cultivars were used, one resistant and one susceptible to *D. noxia*.

Spider Field Collection.—Spiders were hand-collected and numbers counted from the entire area of each plot twice weekly between May–July 2008. The density of spiders was low, so they were pooled into five wheat stages: Zadoks 40, 50, 60, 70, and 80 (Zadoks et al. 1974). *Tetragnatha laboriosa* was sampled between 07:30–09:00, when dew allowed for easy web detection and at a time that corresponds to increased feeding (Culin & Yeargan 1982). *Pardosa sternalis* was sampled between the hours 07:30–9:00 or 13:00–15:00. Individual spiders were transferred into 1.5 mL microcentrifuge tubes filled with chilled 100% ethanol and transferred to the laboratory in a cooler (≤ 4 °C). *Tetragnatha laboriosa* females were difficult to identify without epigynal dissections, which could contaminate the specimens. Thus, they were grouped into an "immature/female" category. *Tetragnatha laboriosa* was not recorded after 19 June 2008, by which time *P. sternalis* was scarce, so the collection of both spider species was discontinued after this date.

Aphid Sampling.—The mean density of *D. noxia* on wheat tillers was estimated by removing all wheat tillers from a random 14 cm^2 area between two of the four aphid-infested rows every two weeks from each replicate of the 0×, 1× and 10× plots, for a total of five dates. Tillers were cut and removed at ground level, placed into a 3.8 L plastic bag, and held on ice until they were transferred into Berlese funnels for 24 h (Tragardh 1933). Aphids were subsequently extracted into 75% ethanol for long-term storage and counting.

Spider Feeding Experiment.—A laboratory feeding study was performed to validate the detectability of *D. noxia* DNA within spiders following consumption. *Pardosa sternalis* were collected alive from dry pitfall traps set in winter wheat adjacent to the aforementioned plots. *Tetragnatha laboriosa* spiders were collected with aspirators from the same adjacent wheat areas. Spiders were maintained in 100 × 15 mm Petri dishes with a moist Plaster of Paris substrate for water supply on a L16:D8 cycle with fluctuating day (24 °C) and night (20 °C) temperatures (Lab-Line Biotronette Plant Growth Chamber, Lab-Line Instrument, Inc., Melrose Park, Illinois, USA), conditions comparable to those observed in the field. Moisture was provided by spraying the inside of each dish twice daily with water. Spiders were fed two to three *Drosophila melanogaster* Meigen 1830 (Diptera: Drosophilidae) every other day for approximately two weeks to reduce stress and maintain the health of the spider prior to the start of the experiment. Spiders were starved for approximately 7 d, and then fed one *D. noxia* biotype 2 aphid. The spiders were individually observed to feed and were stored at −20 °C in 100% ethanol at the following post-feeding times (in h): 0 (i.e., immediately after feeding), 4, 8, 12, 16, and 24, with eight individuals represented for each time period. Spiders were maintained in the plant growth chamber during their digestion period before freezing. Eight starved spiders of each species served as negative controls.

Primer Design.—An 1146 bp sequence of the mitochondrial cytochrome c oxidase subunit I (COI) gene from *D. noxia* was retrieved from the GenBank database (Accession #FJ232620). This, sequences from *P. sternalis* and *T. laboriosa* (sequenced from the universal primers C1-J-1718 and C1-N-2191 (Simon et al. 1994)), and those of the following aphid species derived from GenBank: *Diuraphis frequens* (Walker 1848) (Accession #FJ232622), *D. tritici* (Gillette 1911) (Accession #FJ232621), *Rhopalosiphum padi* (Linnaeus 1758) (Hemiptera, Aphididae) (Accession #AY594671) and *R. maidis* (Fitch 1856) (Accession #AY594673) were aligned using ClustalW (Larkin et al. 2007) within the BioEdit sequence alignment editor (Version 7.0.5, Tom Hall, Ibis Therapeutics, Carlsbad, California, USA). A pair of primers (RWACOIF-F: 5′-CACTTATTA-TGTAGTAGCACATTTTCAT-3′; RWACOIR-R: 5′-TTA-GGATAATCTGTATATCGTCGTGGT-3′) amplifying a 227 bp sequence, were designed using Primer 3 software (Version 2.2.3, S. Rozen & H. Skaletsky,Whitehead Institute, Cambridge, Massachusetts, USA and Howard Hughes Medical Institute, Chevy Chase, Maryland, USA), analyzed with Oligo Analyzer (Version 3.1, Integrated DNA Technologies, Inc., Coralville, Iowa, USA), and optimized by performing a

Table 1.—Arthropods tested with *Diuraphis noxia* primer pairs.

Order	Family	Species
Acari	Tetranychidae	*Oligonychus pratensis* (Banks 1912), *Petrobia latens* (Müller 1776)
Araneae	Gnaphosidae	*Drassyllus nannellus* Chamberlin &Gertsch 1940
	Lycosidae	*Schizocosa mccooki* (Montgomery 1904)
	Thomisidae	*Xysticus pellax* O.P.-Cambridge 1894
Coleoptera	Carabidae	*Bembidion quadramaculatum* (Linnaeus 1761), *Poecilus* sp.
	Coccinellidae	*Coccinella septempunctata* Linnaeus 1758, *Hippodamia convergens* Guérin-Méneville 1842, *Hippodamia parenthesis* Say 1824, *Coccinella transversoguttata* Faldermann 1835, *Scymnus* sp.
Collembola	Isotomidae	Undetermined sp.
Diptera	Culicidae	*Culex pipiens* L. 1758, *Culex tarsalis* Coquillett 1758
	Tachinidae	*Phasia* sp.
Hemiptera	Anthocoridae	*Orius* sp.
	Lygaeidae	*Nysius* cf. *raphanus* Howard 1872
	Miridae	*Lygus* sp.
	Nabidae	Undetermined sp.
	Pentatomidae	Undetermined sp.
	Rhopalidae	*Arhyssus lateralis* (Say 1825)
Homoptera	Aphididae	*Acyrthosiphon pisum* Harris 1776, *Diuraphis frequens*, *Diuraphis tritici*, *Rhopalosiphum padi*, *Rhopalosiphum maidis*, *Schizaphis graminum* (Rondani 1852), *Sitobion avenae* (Fabricius 1775), *Sipha elegans* del Guercio 1905
Thysanoptera	Thripidae	*Anaphothrips obscurus* (Muller 1776)

gradient PCR and by adjusting reagent concentrations, number of cycles, and the denaturation, annealing, and extension times.

DNA Extraction.—The whole-body extraction of DNA from spiders was performed with Qiagen DNeasy Animal Tissue kits (Qiagen, Valencia, California, USA) following the manufacturer's animal tissue protocol. The DNA concentration from the extractions was quantified with a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA), using 1 μL of template. The ratio of sample absorbance at 260 and 280 nm was used to assess the purity of the DNA. DNA concentrations from the spiders ranged from 50–450 ng/μL. DNA concentrations from single aphids ranged from 1–6 ng/μL. After measurement, total spider DNA extractions were diluted to 50 ng/μL for standardization and stored at −20 °C.

PCR amplification and purification.—PCR reactions using the RWA-speeific primers (25 μL) included the following reagents: 2.5 μL of Takara 10× Buffer (100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$), 1.0 μL of each primer (0.4 μM), 2 μL of Takara dNTP mixture (dATP, dCTP, dGTP, dTTP, 2.5 mM of each), 0.625 units/μL of Takara *Taq* HS DNA polymerase (0.125 μL), and 5 μL of template DNA. The PCR protocol included the following: an initial denaturation step of 3 min at 94 °C; followed by 35 cycles of denaturing for 30 s at 94 °C, annealing for 30 s at 60 °C, and extension for 60 s at 72 °C; and a final extension step of 72 °C for 5 min. PCR products were separated by electrophoresis in 2% agarose gels (Fisher Scientific, BP160-500, Pittsburgh, Pennsylvania, USA), post-stained with ethidium bromide for 1 h, and photographed under UV light (Syngene Blue Light Transilluminator, Syngene, Frederick, Maryland, USA). Positive and negative *D. noxia* controls were included in each PCR experiment.

The PCR product from one *D. noxia* (positive control) was purified with a Mo Bio Ultraelean Purification Kit (MO BIO Laboratories, Inc., Carlsbad, California, USA) following the manufacturer's protocol and sequenced following the dideoxychain-termination method at the University of Washington's High-Throughput Sequencing Solutions. The nucleotide identity for both primer pairs matched 100% with *D. noxia*, indicating that the correct region was amplified for PCR.

Cross-reactivity testing.—Extensive primer specifieity testing was conducted to eliminate occurrence of false positives due to the cross-reactivity of primers. Non-target prey (one individual each) were collected from the wheat field where sampling occurred and also from other laboratories and placed in 100% ethanol (Table 1). DNA from these individuals was extracted as described above, and PCRs were conducted with the *D. noxia*-specific primers to ensure that the DNA of these non-target species did not amplify with these primers. The non-target prey were not amplified by the selected primers.

Statistical Analyses.—Molecular half-lives, i.e., the time at which half of the predators are positively identified with prey DNA following consumption (after Greenstone & Hunt 1993), were calculated for each species using the "probit" procedure in SAS (SAS Institute 2002–2008). Frequency tabulations were performed using the "PROC FREQ" procedure in SAS (SAS Institute 2002–2008) with the Chi-Square statistic and associated probabilities to determine whether the percentage of field spiders positive for the presence of *D. noxia* DNA was associated with aphid infestation level, aphid-resistant cultivars or wheat stage. Data from sampled plots were analyzed for *T. laboriosa* for the effects of wheat stage, infestation level, and level of resistance using the "Mixed" procedure in SAS (SAS Institute 2002–2008) with the REML estimation method and the Kenward-Roger approximation for degrees of freedom (Kenward & Roger 1997). Repeated measures models with autoregressive errors and unequal variances across dates were evaluated and used when justified by Akaike's Information Criterion (Burnham & Anderson 2002), which are used to measure the quality of fit. Density analyses were performed for *Pardosa sternalis*, but only densities during wheat stages

Table 2.—Mean number of *Tetragnatha laboriosa* per wheat stage and infestation level, Fort Collins, Colorado, 2008. Means within a column followed by the same capital letters are not significantly different and represent differences between wheat stages within each infestation level. Means within rows followed by the same lower case letters are not significantly different and represent differences between infestation levels at each wheat stage. Means averaged over resistance; 0×,1×, and 10× refer to the respective aphid infestation levels.

Wheat Stage (Zadoks)	Infestation level		
	0×	1×	10×
40	0.00 Aa	0.00 Ba	0.00 Ba
50	0.00 Aa	0.06 Ba	0.06 Ba
60	0.13 Ab	1.19 Ab	1.69 Aa
70	0.06 Aa	0.31 Ba	0.13 Ba
80	0.00 Aa	0.00 Ba	0.00 Ba

were used for analyses due to its high mobility and varied collection times. *T. laboriosa* densities were square-root transformed (x + 0.5) for the density analyses and pooled into the following five wheat stages: Zadoks 40, 50, 60, 70, and 80. When significant effects were observed ($P \leq 0.05$), Tukey-adjusted pairwise comparisons were performed. Raw means are given in the tables and figures presented herein.

For *D. noxia* densities, mixed models with autoregressive errors and unequal variances across dates were eonsidered. Aphid densities were log transformed [$\log_{10}$ (x)]. A model was selected based on the lowest AIC value, and restricted maximum likelihood (REML) was used as a method for estimating the parameters of the model (SAS Institute 2002–2008). A mixed model with an autoregressive order 1 covariance structure with heterogeneous variances across dates (ARH(1)) was ehosen as the appropriate model.

RESULTS

Field collected spiders.—*Tetragnatlia laboriosa:* Sixty-four *T. laboriosa* were eollected in 2008. Of these, 3% were male, 53% were immature (22% penultimate males), and 44% were either female or immature. Tetragnathids appeared at Zadoks 50, peaked at Zadoks 60, and declined at Zadoks 70 and 80. The immatures were assumed to be *T. laboriosa,* as no other *Tetragnatha* species were present at this site (L.M. Kerzicnik, unpublished data). *Tetragnatlia laboriosa* densities were significantly higher at Zadoks 60 than any other wheat stage ($F_{4,\ 217} = 43.70$, $P < 0.0001$) and subsequently declined after this stage. For infestation level, *T. laboriosa* densities differed ($F_{2,\ 217} = 12.08$, $P < 0.0001$) and were higher in the 1× ($t_{217} = -3.93$, $P = 0.0001$) and 10× ($t_{217} = -4.52$, $P < 0.0001$) infestation levels compared to the 0× infestation level. Similarly, *T. laboriosa* densities were affected by wheat stage and infestation level combined ($F_{8,\ 217} = 8.90$, $P < 0.0001$). The highest mean spider density occurred during Zadoks 60 at the 10× aphid infestation level, and densities were higher at the 10× infestation level compared with the 1× ($t_{217} = 2.99$, $P = 0.0031$) and 0× ($t_{217} = -9.33$, $P < 0.0001$) infestation levels at this stage. No significant differences among wheat stages were found at the 0× infestation level (Table 2), and wheat resistance did not affect densities of *T. laboriosa* ($F_{1,\ 211} = 1.67$, $P = 0.1974$).

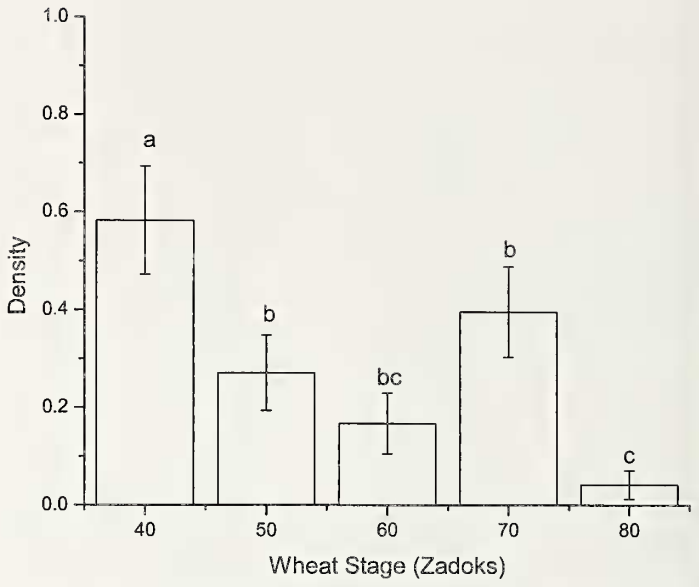

Figure 1.—Mean density (± SE) of *Pardosa sternalis* per wheat stage (Zadoks), averaged over wheat varieties and infestation levels, Fort Collins, Colorado, 2008. Columns marked by the same lower case letters are not significantly different.

Pardosa sternalis: Seventy-one *P. sternalis* were collected in 2008. Of these, 28% were male, 51% were immature, and 21% were female. The immatures collected were assumed to be *P. sternalis,* as no other *Pardosa* were present at the field site (L.M. Kerzicnik, unpublished data). Wheat stage affected the number of *P. sternalis* collected ($F_{4,\ 277} = 7.43$, $P < 0.0001$) with mean densities highest at Zadoks 40 (Fig. 1).

Aphids.—Aphid densities varied with the level of resistance ($F_{1,\ 51.6} = 79.99$, $P < 0.0001$), with susceptible treatments supporting considerably more aphids than the resistant treatments (Table 3), although there was also an interaction between date and resistance ($F_{4,\ 70.1} = 5.74$, $P = 0.0005$). Aphid densities also differed between infestation levels ($F_{2,\ 18.9} = 595.96$, $P < 0.0001$), with the highest aphid densities at the 10× infestation level and densities within the 0× treatments remained close to, or at, zero throughout the experiment. The density of aphids varied with sample date ($F_{4,\ 71} = 208.78$, $P < 0.0001$), with densities peaking on June 4 and then declining in all treatments subsequently (Table 3). There was an interaction between date and infestation level for aphid density ($F_{8,\ 86.8} = 37.84$, $P < 0.0001$). The 10× infestation level was higher than the 1× level, averaged over the level of resistance on May 4 ($t_{32.21} = -6.36$, $P < 0.0001$), May 21 ($t_{34.5} = -5.74$, $P < 0.0001$), and June 4 ($t_{41.8} = -3.74$, $P < 0.0001$). On May 21, June 4, and June 18 there was a difference between aphid densities in the resistant and susceptible treatments, averaged over infestation levels, respectively ($t_{27.2} = -4.76$, $P < 0.0001$), ($t_{38.5} = -4.62$, $P < 0.0001$), and ($t_{31.8} = -6.59$, $P < 0.0001$). An interaction between wheat stage by resistance by infestation level was observed for aphid density ($F_{8;\ 85.9} = 4.04$, $P = 0.0014$) (Table 3). The highest aphid densities oceurred at the 10× susceptible treatment at Zadoks 50, and densities within the 0× resistant and susceptible treatments remained close to, or at, zero throughout the experiment.

Spider feeding experiment.—Results of the spider feeding experiment showed that 100% of *T. laboriosa* tested positive

Table 3.—Mean density of *Diuraphis noxia* on wheat tillers (cm^{-2} d^{-1}) at the 1× and 10× infestation levels for resistant (R) and susceptible (S) wheat lines in Fort Collins, Colorado, 2008. Means within a column followed by the same capital letters are not significantly different and represent differences between dates at each treatment. Means within rows followed by the same lower case letters are not significantly different and represent differences between treatments at each date and wheat stage.

Date	Wheat Stage (Zadoks)	0×R	0×S	1×R	1×S	10×R	10×S
4 May	30	0.00Bc	0.00Cc	0.19Cb	0.38Cb	0.86Ca	1.16Ca
21 May	40	0.00Bd	0.01Cd	1.89Be	3.94Bb	5.04Bb	12.27Ba
4 June	50	0.10Ad	0.22Bd	8.35Ac	16.86Ab	17.64Ab	59.74Aa
18 June	70	0.01Bc	0.49Ac	7.49Ab	14.05Aa	6.35Bb	19.87Ba
2 July	80	0.02Bb	0.20Ba	0.19Ca	0.23Ca	0.20Ca	0.22Da

for *D. noxia* immediately after feeding and declined thereafter such that no predators were positive after 12 h. The molecular half-life was calculated as 4.2 ± 1.1 (SD) h. Similarly, 100% of *P. sternalis* tested positive for *D. noxia* DNA immediately after feeding but detection declined rapidly thereafter, indicating rapid degradation of this primer region in these predators. The molecular half-life for *D. noxia* DNA in *P. sternalis* was calculated as 2.0 ± 0.4 (SD) hrs. Starved predators always screened negative.

Predation and Gut-Content Analysis.—*Tetragnatha laboriosa:* Of the 64 *T. laboriosa* collected from all wheat stages and infestation levels, 32.8% were positive for the presence of *D. noxia*. The number of spiders testing positive for the presence of *D. noxia* DNA was not significantly related to wheat stage (X^2_3 = 3.18, P = 0.37), infestation level (X^2_2= 1.61, P = 0.45), or between aphid-resistant cultivars (X^2_1= 0.22, P = 0.64).

Pardosa sternalis: Of the 71 *P. sternalis* collected, 48% were positive for the presence of *D. noxia* DNA. The number of spiders testing positive for the presence of *D. noxia* DNA was not significantly different between wheat stages (X^2_4 = 6.08, P = 0.19) or resistance levels (X^2_1 = 0.07, P = 0.79). However, as aphid infestation level increased from 0× to 10×, the percentage of spiders testing positive for *D. noxia* increased significantly (X^2_1 = 8.91, P = 0.0028) (Fig. 2).

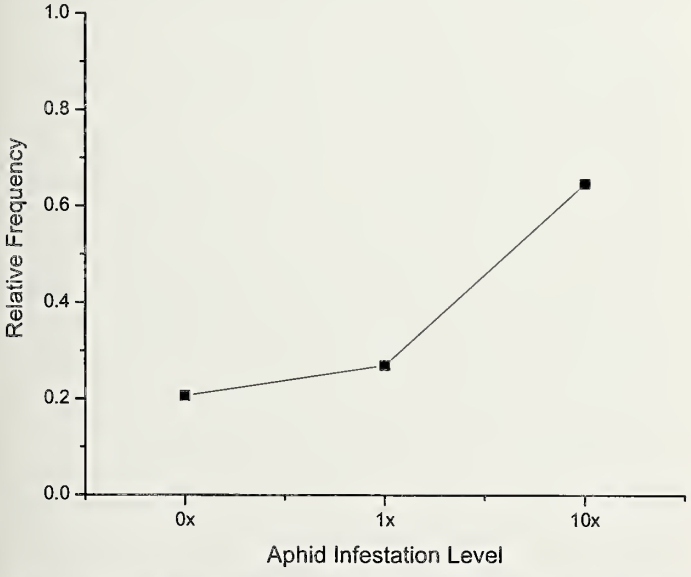

Figure 2.—Relative frequencies of *Pardosa sternalis*, averaged over wheat varieties and wheat stages, testing positive for the presence of *Diuraphis noxia* DNA, Fort Collins, Colorado, 2008.

DISCUSSION

Conservation biological control requires a fundamental understanding of the role natural enemies play in agroecosystems and the temporal dynamics of their populations. This study examined the relationship between two abundant predators, *T. laboriosa* and *P. sternalis*, and a major pest of winter wheat, *D. noxia*, by testing the hypothesis that both spider density and predation would increase with increasing aphid infestation level within resistant cultivars. Most importantly, both predators frequently screened positive for the presence of *D. uoxia* DNA (33% and 48%, respectively), indicating that significant levels of pest consumption occurred under natural field conditions.

Over 92% of *T. laboriosa* were collected at the 1× or 10× aphid infestation levels, and the majority were present within the 10× infestation level, demonstrating some level of aggregation to *D. noxia*. Also, the abundance of *T. laboriosa* peaked at Zadoks 60, the growth stage that occurred between the two highest *D. noxia* densities, and was highest once again within the 10× aphid infestation level. *Tetragnatha laboriosa* also showed evidence for residing in, and constructing webs where, aphid densities were highest, a phenomenon also reported as occurring with linyphiid spiders in alfalfa (Romero & Harwood 2010). This spider balloons throughout its lifetime (Bell et al. 2005), which is an important trait to possess when residing within agroecosystems that are characterized by frequent disturbances. Indeed, the rapid recolonization of highly disturbed agricultural habitats is critically important in biological control (Welch et al. 2011), and these combined attributes are optimal for effective biological control and further indicate an association with the pest.

Pardosa sternalis densities were highest at Zadoks 40 and Zadoks 70, and consumption of *D. noxia* was also highest at these times. Interestingly, a concurrent study within the same treatments found that the falling rate of *D. noxia* was significantly higher in the resistant cultivars compared with its susceptible counterparts at the 10× aphid infestation level at both of these wheat stages (Kerzicnik et al. 2010). Thus, *P. sternalis* is likely utilizing the aphid prey source when it is available.

Biological control is most efficient when generalist predators are present in the crop early before pests reach peak densities (Edwards et al. 1979; Ekbom & Wiktelius 1985; Chiverton 1986; Birkhofer et al. 2008). *Pardosa sternalis* was most abundant at Zadoks 40, prior to peak aphid densities, and demonstrated a high aphid consumption rate, traits characteristic of successful biological control agents. Additionally, the

cursorial spiders *Xysticus cristatus* (Clerck 1757) (Araneae: Thomisidae) and *Pardosa palustris* (Linnaeus 1758) (Araneae: Lycosidae) suppressed growth of the English grain aphid, *Sitobion avenae* (Fabricius 1775) (Homoptera: Aphididae) (Birkhofer et al. 2008). This contrasts with *T. laboriosa*, which colonized wheat fields at peak aphid densities and dispersed to an adjacent corn crop as *D. noxia* densities declined by over 97% from Zadoks 70 to Zadoks 80 (L.M. Kerzicnik, pers. obs.). Therefore, it is important to understand agricultural management practices that encourage the early colonization of generalist predators such as *P. sternalis* to prey on pests at lower densities.

The retention time of target DNA for both *T. laboriosa* and *P. sternalis* was particularly short (4.2 h and 2.0 h, respectively). This contrasts to a number of other studies; for example, collembola DNA has been detected from within linyphiid (Araneae: Linyphiidae) spider guts for up to 24 h post feeding (Agustí et al. 2003). Short retention times, however, are not always disadvantageous when studying predator-prey interactions (Sheppard & Harwood 2005). A shorter retention time can provide detailed information about a recent predation event, and in a study tracking the predation of *Rhopalosiphum padi* by *Pardosa*, the molecular half-life was only 3.7 h (Kuusk et al. 2008). Although physiological and morphological differences could also play a part, predator to prey size ratio can be important for detection of prey material. Using monoclonal antibodies and ELISA, pink bollworm eggs, *Pectinophora gossypiella* (Saunders 1844) (Lepidoptera: Gelechiidae), were detected longer from inside the guts of a minute pirate bug, *Orius insidiosus* (Say 1832) (Hemiptera: Anthocoridae), than a coccinellid beetle, *Hippodamia convergens* Guérin-Méneville 1842 (Coleoptera: Coccinellidae) (Hagler & Naranjo 1997). Both *P. sternalis* and *T. laboriosa* were approximately five times larger than a single *D. noxia* prey. Other studies that indicate longer retention times with prey DNA in feeding trials were more representative of a smaller predator: prey size ratio (Agustí et al. 2003; Monzó et al. 2010). Nevertheless, predation rates were high for both species in the field, validating the approach used here and suggesting very high levels of pest consumption by these spiders during diurnal hours. Aphid prey were also more readily available at this time, and other studies in alfalfa indicated significantly higher prey availability for linyphiid spiders at night (Romero & Harwood 2010).

Although spiders are unlikely, alone, to reduce pest densities below economically damaging levels, synergism could take place with multiple predators with divergent foraging strategies to allow for increased predation (Losey & Denno 1998; Schmidt et al. 2003). Given the high rates of predation on *D. noxia* for both spiders, it is likely that *T. laboriosa* can capture many aphids within the above-ground wheat while *P. sternalis* can intercept aphids on the ground prior to re-colonization of the crop, identifying an important predatory component for pest management.

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